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The Role of the Male-produced Pheromone in the  
Reproduction Behaviour of the Southern Armyworm  
Pseudaletia separata (Wlk.)

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requirements for the degree of Master of Science  
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## Introduction

Detailed courtship patterns have been described for few species of Lepidoptera. Most of the descriptive work in the literature is fragmentary or lacking in experimental or statistical analysis. Brower, Brower, and Cranston (1965) working on wild populations of the queen butterfly Danaus gilippus berenice have statistically analysed the probability of each movement in the sequence and have given a reliable and detailed account of mating in this species. Tinbergen (1958) by the presentation of models of varying size and colouration, and examining the affect of removal of structures suspected to be important in courtship has produced a good experimental account of mating in Satyrus semele the grayling butterfly. Because of the greater difficulties of observing complete mating sequences of nocturnal insects in the wild, most moth studies have been carried out with small caged populations. Again detailed experimental work is rare. The studies of Shorey (1964) on the cabbage looper Trichoplusia ni and Birch (1970) on the angleshades moth Phlogophora meticulosa possess good experimental detail suggesting that chemical cues are much more important in the courtship of moths than in butterflies.

Development of more definitive techniques has allowed the investigation of insect courtship to move from subjective descriptions of the movement sequence to precise studies of the visual, tactile and chemical cues. In particular the availability of gas chromatography and mass spectrometry techniques capable of detecting the very small amounts of material produced by insects, has revolutionised the study of the chemicals, or pheromones, that are used in intra-specific communication.

Pheromones are very widespread amongst the insect orders, and have a variety of functions in addition to sexual attraction. Many families of the Lepidoptera have had pheromones implicated in courtship eg. the Bombycidae Butenandt (1963), the Lymantriidae Jacobsen, Beroza and Jones (1960) the Noctuidae Gaston Fukuto and Shorey (1966) and several others. Female cockroaches produce volatile substances which attract males and induce typical precopulatory behaviour, Barth (1961). Coppel, Cassida and Dauterman (1960) have shown the presence of a potent attractant in virgin females of the sawfly Diprion similis (Hymenoptera). Males of Musca domestica, the common housefly are attracted to the female by a pheromone Rogoff (1964). These examples are a few selected from the immense literature on this subject. Other functions of pheromones are eliciting alarm reactions in bees and ants, trail following in ants and depression of ovary development in worker bees Regnier and Law (1968).

The Noctuidae have attracted considerable attention largely because they are a widespread group of considerable economic importance. Gaston, Shorey and their co-workers have carried out an intensive study of the male attracting pheromone produced by females of Trichoplusia ni. They have discussed the environmental control of mating Shorey (1966), the bioassay of the pheromone Gaston and Shorey (1964), pheromone isolation techniques Gaston Fukuto and Shorey (1966) circadian rhythm of pheromone responsiveness Shorey and Gaston (1964) quantitative aspects of the production and release of the pheromone with respect to age and mating history Shorey and Gaston (1965) Shorey, M<sup>C</sup>Farland and Gaston (1968) and described the morphology of the female gland Jefferson, Shorey and Gaston (1965) Miller, Jefferson and Thomson (1967). Many other much less detailed studies of the female pheromone of different noctuid species exist.

Very much less is known about the pheromones produced by the males of this family. Because of the difficulty of detecting an overt behavioural response from the female when stimulated by the pheromone of a conspecific male, the function of these pheromones is not at all clear. On the basis of unsophisticated preliminary experiments Shorey (1964) concluded that none of the observed female responses were invoked by any pheromone from the external tufts of brown hair on the males abdomen in T. ni. Electrophysiological evidence from Grant (1970)

demonstrated that the female does detect substances from these hair tufts, though no indication of function has yet been given. In other Lepidopteran families, the function of the male pheromone is clear. N - undecanal the male pheromone of Galleria mellonella is an attractant stimulating the female to move towards the displaying male Roller, Bieman, Bjerke, Norgard and M<sup>C</sup>Shan (1968). In contrast the pyrolizine of Danaus gilippus berenice is an arrestant, arresting the flight of the female and inhibiting her from flying away from the male once she has been induced to alight Brower, Brower and Cranston (1965) Pliske and Eisner (1969).

Structurally, the complex organs producing the male pheromone in the Noctuidae are diverse, including the hair pencils of Persectania avera, the wing glands of Erana graminosa, the giant posterior brush of Plusia chalcites and the tibial hairs of Dasypodia selenophora. The only common feature is that all appear to have evolved from modified scales and the surrounding cells. This great variation in structure and location would seem to indicate that pheromone producing structures have evolved indepenantly many times in response to a powerfull selection pressure. The first accurate description of the noctuid hair pencil was the work of Stobbe (1912). This worker described the hair scales, everting structures, and the hair pouch. His assertion that a gland consisting of a small number of very large cells is the major secretory structure has supplanted the theory of Eltringham (1925) that scales in the bottom of the hair pencil pouch produce the pheromone.

This thesis is a detailed examination of the mating sequence of the noctuid Pseudaletia separata (Wlk.) with particular reference to the chemistry and function of the male pheromone and of the cytology and physiology of the secretory structures.

### The Mating Sequence.

Before strict experimental analysis can be carried out, a series of preliminary observations must be made to compile an action sequence for the species. In insects behaviour patterns are totally inherited, the major exceptions being the social species. These inherited patterns may seem very rigid e.g. the form of the cases of the caddis fly larva Mollanna sp is species specific with little individual variation in a natural population. When conditions are altered however, these insects show a adaptability which would not be expected from a chain of unconditioned reflexes Carthy (1965). Because of this adaptability, interpretation of behaviour under laboratory conditions must proceed with caution.

Methods:- The most critical condition for the observation of a nocturnal moth would appear to be lighting conditions. Shorey (1966) found that the response of male Trichoplusia ni was greatly inhibited at light intensities greater than one lux. Compared with the mamalian eye, the visible spectrum of the insects compound eye is shifted towards the ultra violet end. Burkhardt (1964) states "The bee does not react to wavelengths above about 600 m u nor do most other insects investigated unless extreme intensities are applied".

For this study, a light was produced with no emission of wavelengths below 600 m u (checked with Hitachi recording spectrophotometer). Moths of this species showed definite changes in flight pattern near this light source and its use was discontinued. Other noctuids have also been shown to detect red wavelengths e.g. Birch (1970) found that males of Plusia gamma orientate toward direct light from a red bulb in preference to a receptive female. Distant fluorescent light were used as the light source in this study. By comparison with the accurately measured light intensity of a variac controlled bulb, it was estimated that the experimental light intensity was less than one lux.

It was not found necessary to provide artificial air movement as mating occurred readily in the almost completely enclosed glass cages. In contrast males of Phlogophora meticulosa were unable to orientate to females in still air Birch (1970).

The other experimental conditions were not unusual. Seven to nine pairs of virgin moths from a laboratory culture were placed in a glass observation cage, with an internal volume of  $2.8 \times 10^4 \text{ cm}^3$ , immediately following emergence. A solution of 10% sucrose was continuously available on a wad of cotton wool, suspended from the roof of the cage. Temperatures were in the region of 20°C.

Results:- The observed sequence was not complex.

After a period of sexual display the male located the passive female and mated.

The first overt sexual action of the male was the rhythmical extrusion and retraction of the external genitalia while at rest on the side of the cage. The number of males active in this way increased, till about an hour later this display gave way to active flight with the genitalia continuously extruded. These males approached females from below and behind, while curving the abdomen upward so that the external genitalia approached the abdominal tip of the female (see fig 1). Exsertion of the hair pencils was not observed. From this position, which was held for upwards of a second, the male made an upward turning snatch, clasping the tip of females abdomen with the valvae. The whole sequence of coupling from approach to completion rarely required more than two seconds. After coupling the male hung downwards and the pair moved steadily towards the floor. The male appeared to be the active partner in this descent, with the female passively following.

Courtship by the male always appeared to be directed toward females. Only on one occasion was a displaying male observed to pass close to another male, but it did not orientate itself toward the second male in any way.

Successful mating occurred only with females resting motionless on a vertical surface, or on the lower side of a horizontal surface. The posture of the female was variable. Sometimes the female was observed to assume a "calling" position. The costal margin of the wings was strongly depressed, the thorax and wings forming a triangle while the abdomen was markedly elevated. The posture of other females was indistinguishable from that of resting moth. Most of the observed unsuccessful matings were due to the approached moth walking or flying away





FIG 1 THE MATING APPROACH

before contact was made. One male was observed to clasp another moth as it flew past. The clasped moth beat its wings strongly and the pair separated about a minute after initiation of contact.

The majority of matings were observed to occur between 10:00 p.m. to 11:30 p.m.

Conclusions:-The observed mating sequence of Pseudaletia separata contained no behavioural elements not already described for other species.

Comparison of the mating sequence of Trichoplusia ni Shorey (1964) Grant (1970) and Phlogophora meticulosa Birch (1970) with Pseudaletia separata revealed several differences in the precopulatory behaviour of the males. During the pre-copulatory flight, P. meticulosa and P. separata were observed to evert the external genitalia frequently, an action seen in T. ni only just before copulation. To locate the female, both T. ni and P. meticulosa need to orientate to an airstream while in flight, a requirement not seen in P. separata. The initial contact with the female is tactile in T. ni (antennae and tarsi) and P. meticulosa (antennae only) while P. separata appeared not to touch the female with either. In all three species, the external genitalia are extruded and orientated toward the abdominal tip of the female. The clasping of the female which follows, occurs while the male hovers beside (T. ni) or below (P. separata) the female, while in P. meticulosa

the male must land before the genitalia are engaged.

P. meticulosa and P. separata possess anterior hair pencils, while T. ni has a pair of scent brushes near the external genitalia. These scent brushes appear and are spread briefly just before mating Grant (1970). Similarly Birch (1970) reports the eversion of the hair pencil following orientation of the external genitalia toward the female. The failure to observe a similar eversion of the hair pencils of P. separata may be due inadequate experimental conditions. The density of the experimental population may have been too high, or the light intensity may have been too high. The structure is most unlikely to be vestigial, as several individuals from a wild population sampled with a light trap had the hair pencils everted and folded between the thorax and abdomen, or more rarely, hanging loosely. Birch (1970) demonstrated the hairpencil eversion in P. meticulosa is obligatory for successful copulation. From the description of Grant (1970) it appears that spreading of the brushes does not occur in all successful matings of T. ni. Other species eg. Spilosoma lubricipeda (Arctiidae) and Deilephila elpenor (Sphingidae) mate in the laboratory without coremata or hair pencil eversion Birch (1970). It is thus concluded that in P. separata and some other species, hair pencil eversion is facultative, while in other species eg., P. meticulosa this behavioural element is obligatory.

The "calling" attitude of the Lepidopteran female appears to be adopted in order to disperse a pheromone, a suggestion first

made by Poulton (1928). This attitude is reported from many families eg, Sanninoidea exitiosa (fam. sessidae) Smith (1965) Anagasta kuhniella (fam. Phycitidae) Richard and Thompson (1932) and Protoparce sexta (fam. Sphingidae) Allan and Hodge (1955). The posture of females of T. ni appears to be identical to that of P. separata. The eighth and ninth abdominal segments usually retracted within the seventh are extruded, Shorey (1964) exposing the modified intersegmental membrane that produces the pheromone. Jefferson Shorey and Gaston (1966). As males of P. meticulosa respond to artificially everted glands at any time, Birch (1970) suggests that eversion of the female gland is the event determining the species specific time of mating.

A mated pair would be very vulnerable to predation due to the difficulty of movement while paired and the conspicuous nature of the behaviour that preceded mating. Brower, Brower and Cranston (1965) suggest that the function of the post nuptial flight in Danaus gilippus berenice is to carry the pair away from where they have been so conspicuously active to a less obvious area. By analogy, a similiar function is suggested for the downward movement of mated pair observed in P. separata.